CHARACTERIZATION OF THE TWO LARGE FRAGMENTS FROM PEPSIN-DIGESTED DENATURED COLLAGEN

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The purification of the fragments, designated α' and D, is described in the previous abstract (Lampiaho, K. et al.).

Sedimentation velocity studies in acetate buffer, pH 4.8, ionic strength 0.15, at 38°C resulted in $S^{\circ}_{20,w}$ = 2.66 S for α' and 1.95 S for D. Molecular weights calculated according to Williams et al. (J. phys. Chem. 58, 774, 1954) were 61,500 for α' and 28,200 for D (assuming M = 95,000, $S^{\circ}_{20,w}$ = 3.20 S for the α-, and M = 190,000, $S^{\circ}_{20,w}$ = 4.34, for the β-component of tropocollagen).

Amino acid analyses (Spackman, D. H. et al.: Anal. Chem. 30, 1190, 1958) gave minimum molecular weights of 60,500 for α' (1 His, 2 Hylys) and 29,900 for D (1 Met, 2 His, 2 Hylys). The over-all amino acid composition is typical of collagen, but definite differences exist between α' and D, *e.g.*, higher Hypro:Pro ratio and excess of basic residues in D. Dinitrophenylation of α' gave a quantity of N-terminal amino groups corresponding to M = 59,000. Cleavage of α' with CNBr resulted in three bands on starch gel electrophoresis in accordance with the 2 methionyl residues in α'.

Fingerprinting of the tryptic hydrolysates of both α' and D showed that D cannot be included into α', nor could any fragment corresponding to D be liberated from α' by further digestion with pepsin.

Electron microscopic pictures of the SLS-particles obtained from α' and D after renaturation (Kühn, K. et al.: Arch. Biochem. 190, 534, 1965) proved, that the fragment α' is located in the B end and the fragment D in the A end of the tropocollagen molecule with relative lengths in accordance with the measured molecular weights.